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(21) International Application Number: PCT/DK91/00203 (22) International Filing Date: 12 July 1991 (12.07.91) (30) Priority data: 1687/90 13 July 1990 (13.07.90) DK (71) Applicant (for all designated States except US): NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). (72) Inventors; and (75) Inventors/Applicants (for US only) : JUNKER, Flemming [DK/DK]; Langebjerg 133, DK-3050 Humlebæk (DK). THEISEN, Claus, Friis [DK/DK]; Rønnegade 14, DK-2100 København Ø. (DK). (74) Common Representative: NOVO NORDISK A/S; Patent Department, Novo Allé, DK-2880 Bagsvaerd (DK).		(81) Designated States: AT (European patent), AU, BE (European patent), BG, BR, CA, CH (European patent), CS, DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, MW, NL (European patent), NO, PL, RO, SD, SE + (European patent), SU, US. Published <i>With international search report.</i>
(54) Title: GROWTH HORMONE CRYSTALS AND A PROCESS FOR PRODUCTION OF THESE GH-CRYSTALS (57) Abstract A method of producing chemically stable and biologically active growth hormone crystals and processes for production of pharmaceutical preparations containing these growth hormone crystals.		

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GROWTH HORMONE CRYSTALS AND A PROCESS FOR PRODUCTION OF THESE GH-CRYSTALS

Field of invention

The present invention concerns a method of producing growth hormone crystals in the presence of cations, novel growth hormone crystals and pharmaceutical preparations containing such novel crystals.

Background of the invention

The growth hormones (GH) from man and from the common domestic animals are proteins of approximately 191 amino acids, synthesized and secreted from the anterior lobe of the pituitary. The growth hormone is a key hormone involved in the regulation of not only somatic growth, but also in the regulation of metabolism of proteins, carbohydrates and lipids.

During the past 40 years or more much attention has been devoted to the unravelling of the biochemical function of the growth hormones from various species. The reason for this interest in the molecular function of this protein rests upon the commercial interests from both veterinarian and medical circles. The GH gene has now been cloned and human growth hormone (hGH) and Met-hGH are currently being produced biosynthetically by the use of both bacteria and mammalian cell cultures.

Pharmaceutical preparations of GH tend to be unstable. Degradation products such as deamidated or sulfoxidated products and dimer or polymer forms are generated - especially in solutions of GH. Therefore, today GH is lyophilized and stored in the lyophilized form at 4°C until it is reconstituted by the patient, before start of use.

The reconstituted preparations are preferably stored at 4°C to minimize degradation in solution. However some degradation will take place during such storage which can be for a period of up to about 14 days. There is thus a need in the art for more stable preparations of GH.

It would also be an advantage to avoid the lyophilization step in the production of GH preparations. Lyophilization is a time consuming and costly process and also often a limiting procedure due to the capacity of the freeze drier.

10 The present invention is based on the surprising recognition that the above needs are fulfilled by means of a crystallization step in the production of GH.

Although readily available in quantities sufficient for crystallization, GH has so far eluded successful crystallization. Micro crystals, or amorphous material have been reported
15 from a variety of sources: (Jones et al., Bio-Technology (1987) 5, 499 - 500; Wilhelmi et al., J.Biol.Chem. (1984) 176, 735 - 745; Clarkson et al., J.Mol.Biol. (1989) 208, 719 - 721; and Bell et al., J.Biol.Chem. (1985) 260, 8520 - 8525.

20 The hanging drop method is the most common method in use for this purpose. Apparently due to heterogeneity among growth hormone preparations the size and the shape of the crystals have been reported to vary significantly. The largest crystals have been reported by Jones et al. (1987). For their successful
25 full experiments they used a mixture of polyethylene glycol 3500 and beta octyl glucoside at neutral pH. Clarkson et al. (1989) reported that the use of lower alcohols and acetone permitted the generation of crystals of 0.001 to 0.005 cubic mm with varying shapes. None of the known methods are however
30 suitable for commercial production of GH crystals a.o. due to the fact that growth times of from several weeks up to one year are needed.

Bovine growth hormone has been formulated for veterinarian use in a mixture of divalent ions and an oil (EP 343 696). By addition of $ZnCl_2$ to either bovine or ovine growth hormone in the presence of lipids undefined particles were produced to form a prolonged release formulation. The growth hormone was dispersed in the carrier in such a way as to trap 1 to 4 Zn molecules per growth hormone molecule. The solutions were prepared in the presence of varying concentrations of denaturing solutes (1 to 4 M of urea) at high pH (9.5). A reproduction of this process with hGH has shown that it is not possible to produce crystals in this way.

From the literature it is well known that the presence of divalent cations during the process of crystallization permits not only excellent orientation during analysis, but also improved physical conditions for the crystallization of insulin (e.g. US pat. no. 2174862). Growth hormone is, however, more than three times larger than insulin and has a totally different conformation. Surprisingly the addition of cations to solutions containing hGH have now permitted the generation of stable, uniform crystals of the growth hormone in high yields. Furthermore, the time used for the formation of high quality hGH crystals is relatively short.

Summary of the invention

In its broadest aspect the present invention is related to a process for production of cation crystals of GH or GH derivatives, comprising the following steps:

- a) adding cations of inorganic or organic nature to a solution of GH or derivatives thereof at a pH between 5 and 8,
- b) growing of crystals at a temperature from about 0 to about 30°C, and
- c) isolation of the cation crystals by known means.

In the present context GH is intended to cover all species of GH including human, bovine, porcine, ovine, salmon, trout or tuna. GH derivatives are intended to cover GH of human or animal species with minor variation in the protein sequence.

5 Thus a few amino acid residues may have been deleted or replaced by other amino acid residues. Also covered is truncated forms of growth hormone and derivatives thereof as well as growth hormones with amino acid residues added to the N- and/or C-terminal end of the protein, such as Met-hGH.

10 The process according to the present invention has for the first time made it possible to produce chemically stable and uniform cation-GH crystals. Also, the present process enables production of both larger and smaller crystals of growth hormone, as the need may be.

15 The pH in step a) is preferably from 5.0 to 7.5, more preferably from 5.0 to 6.8, even more preferably from 5.8 to 6.5, and most preferably from 6.0 to 6.5.

According to a preferred embodiment of the present invention the growth hormone is of human nature.

20 The cations may be of inorganic or organic nature. Divalent cations are preferred and of these an inorganic cation such as Zn^{++} has turned out to be well suited for the fast formation of stable GH crystals. Also mixtures of these cations can be used.

The cation should be added in an amount providing fast and
25 efficient formation of well defined crystals. The upper limit for the amount of added cation is the amount which would cause unspecific precipitation of substantial amounts of amorphous material.

If Zn^{++} is used, suitable concentrations will typically be from
30 about 0.2 to 10 mol Zn^{++} /mol GH. However, if the crystallization reaction mixture contains a buffer or other compound which is

capable of binding some of the cation, e.g. in a complexed form, greater concentration of the cation will be needed because some of the cation will not be available for the crystallization process.

5 Zn⁺⁺ will preferably be used in an amount which will cause formation of GH crystals with a molar ratio between Zn⁺⁺ and GH from about 0.2 to about 10, preferably from about 0.5 to about 5 and more preferably from about 0.5 to about 2.

In a preferred embodiment of the invention there is added an
10 organic solvent or a mixture of organic solvents in step a). The organic solvent may be chosen from the group consisting of short chained aliphatic, cyclic or aromatic alcohols and ketones. Suitable organic solvents are acetone, methanol, ethanol and 2-propanol. A preferred organic solvent is ethanol
15 or acetone. The concentration of the organic solvent may be from 0.1 to 50% v/v, preferably from 0.1 to 30%, more preferably from 0.1 to 20%, even more preferably from 5 to 15% and most preferred from 6 to 12% v/v.

The present process may be used as a fast and efficient down
20 stream processing of the growth hormone in question, due to the formation of crystals in large volumes of solutions.

The present invention is also related to novel cationic crystals of GH or GH derivatives.

The crystals are preferably hGH crystals or crystals of
25 derivatives of hGH. The cation is preferably Zn⁺⁺ and the molar ration between Zn⁺⁺ and GH will typically be from about 0.2 to 10, preferably from 0.5 to 5 and more preferably from 0.5 to 2.0. The size of the crystals will be dependent on the Zn⁺⁺ to GH ratio and the choice and content of solvent used in the
30 process.

hGH crystals according to the present invention have been shown to have a biological potency similar to that of a solubilized hGH standard in in vitro and in vivo tests. The novel GH crystals can thus be used for the same indications as the 5 commercially available hGH preparation.

Pharmaceutical preparations containing the novel GH crystals will typically be solutions or suspensions and may contain the usual adjuvants and additives used for pharmaceutical hGH preparations, such as buffers, glycerol and preservatives. The 10 preparations may be administered in the same way as the commercial hGH preparations. The crystals may also be formulated as dried crystals which will then have to be reconstituted before start of use.

The pharmaceutical preparations containing the novel GH 15 crystals have surprisingly a very high chemical stability compared with preparations made from commercially available GH.

The present invention therefore provides for a possibility of production of pharmaceutical preparations that are more convenient, especially for the patients. Due to the high 20 stability of the crystals in suspension, the present invention will as an example make it possible to produce ready to use pharmaceutical preparations in the form of suspensions which will not need to be reconstituted by the patients before use.

In a further aspect the invention provides a valuable tool for 25 production and purification purposes of GH.

Detailed description of the invention

The starting material, the growth hormone that may be of any origin and if desired derivatized in solution, is adjusted to a concentration preferably greater than about 0.1 mg/ml, more

preferably from about 4 to about 7 mg/ml and most preferred about 6 mg/ml. The pH will preferably be from 6.0 to 6.3.

To the above mentioned solution may be added an organic solvent. A preferred organic solvent is ethanol in a concentration which may vary between 0,1 and 20%, preferably 5 and 15%, and most preferred 6 and 12%.

Other solvents such as acetone, methanol or propanol may be used alone or as a mixture instead of or together with ethanol in a concentration within the range of from 1 to 50%.

10 Cations of inorganic or organic nature, or mixtures thereof are then added to the resulting solution.

A preferred cation is Zn^{++} which will normally be used in a concentration from 0.5 to 10 mol/mol GH, preferably from 1.0 to 3.0 mol/mol GH, more preferred from 1.1 to 2.2 mol/mol GH and
15 most preferred from 1.2 to 2.0 mol/mol GH.

If cations of inorganic nature other than Zn^{++} are used, the concentration may be varied between 0.5 and 10 mol/mol GH.

The crystals are then grown for a period of from 1 to 120 hrs. preferably 5-72 hrs., most preferred 20-48 hrs., and at a
20 temperature of between 0 and 30°C, preferably from 4 to 25°C.

The crystals may be recovered by centrifugation or filtration, followed by washing and/or freeze drying to remove remaining organic solvents.

Pharmaceutical preparations of dried crystals or crystals in
25 suspension can now be formulated by using various selected buffers and other pharmaceutically acceptable additives.

The invention is further illustrated but not limited by the following examples:

Example 1Crystallization of hGH in the presence of Zn^{++} .

500 ml of hGH solution produced according to H. Dalbøge et al.,
5 Bio-Technology (1987), 5, 161 - 164, in a concentration of 6
mg/ml was incubated in 10 mM phosphat buffer (NaH_2PO_4) and
adjusted to pH 6.1 with H_3PO_4 . Acetone was added to a final
concentration of 10% (v/v) and thereafter zinc acetate solution
was added to a final concentration of 0.08 mg $ZnAc_2$, $2H_2O$ /ml -
10 1.34 mol Zn^{++} /mol hGH.

The resulting solution was left at 15°C for 20 hours, whereby
crystals were allowed to form.

After this the crystals were recovered and washed 3 times with
crystallization buffer without acetone. The crystallization was
15 checked by microscopy and the size of the crystals were
measured to 8-12 μm . A photomicrograph is shown in Figure 1.

The crystal yield of hGH was determined by solubilization of
the washed crystals in 7M urea followed by ion exchange HPLC
analysis.

20 The yield was found to be more than 50%.

Example 2

Example 1 was repeated with the exception that Met-hGH was used
instead of hGH. The crystals recovered by this process were
identical in shape and size to those obtained with hGH. The
25 yield was more than 50%.

Example 3

Example 1 was repeated with the exception that the addition of acetone was omitted.

The crystals of hGH resulting from this procedure were much 5 smaller than the crystals resulting from Example 1, less than 2 μm .

Example 4

Example 1 was repeated under conditions where acetone was exchanged with ethanol and temperature during growing period 10 was 20°C instead of 15°C. All other experimental conditions were identical to those described in example 1. By varying the ethanol concentration the optimal concentration was found to be 7.5% (v/v). The yield was increased to >80% if the motherfluid following initial crystallization for 16 hrs was supplemented 15 with further 4% (v/v) ethanol and the crystallization temperature was lowered from 20° to 10°C over a period of 16 hrs. The size of the crystals were between 3 to 6 μm with a shape similar to that described in example 1.

Example 5**20 Determination of the amount of Zn bound in hGH crystals**

Example 1 was repeated with the exception that ethanol in a concentration of 7.5% (v/v) was added instead of acetone and that crystals were allowed to form for 16 hrs at 20°C, then the crystals were separated from the motherfluid by centrifugation 25 and washed once with 10 mM phosphate buffer. The crystals were solubilized by raising the pH to 8.0 with NaOH. The hGH was measured by ion exchange HPLC or by UV determination. The Zn concentration was measured by atomic absorption and the results

were compared with those values obtained for the total crystal suspension. The ratio of bound Zn to hGH was found to be 1.9 mole of Zn per mole of hGH.

Example 6

5 Formulation of a Pharmaceutical Preparation Containing hGH:

Crystals were grown as described in example 5 and stored at 4°C. The crystals were then isolated by centrifugation and subsequent removal of the motherfluid. Then the crystals were freeze dried over night to achieve dry crystals with no
10 remaining organic solvent. A pharmaceutical suspension of the dried crystals was prepared according to the following formulation:

hGH crystals	1.3 mg/ml
NaH ₂ PO ₄ ·2H ₂ O	3.0 mg/ml
15 Zn(Ac) ₂ ·H ₂ O	0.1 mg/ml
Glycerol	15.0 mg/ml
Benzyl alcohol	15.0 mg/ml

pH was adjusted to 6.2.

Example 7

20 Example 6 was repeated with the exception that Zn(Ac)₂·H₂O was omitted, giving a suspension of the following formulation:

hGH crystals	1.3 mg/ml
NaH ₂ PO ₄ ·2H ₂ O	3.0 mg/ml
Glycerol	15.0 mg/ml
25 Benzyl alcohol	15.0 mg/ml

pH was adjusted to 6.2.

Example 8

The crystals were treated in the same way as in example 6 and the following suspension was formulated:

hGH crystals	1.3 mg/ml
5 NaH ₂ PO ₄ · 2H ₂ O	2.5 mg/ml
NaCl	5.7 mg/ml
Benzyl alcohol	15.0 mg/ml

pH was adjusted to 6.2.

Example 9

10 The crystals were treated in the same way as in example 6 and the following solution was prepared:

hGH crystals	1.3 mg/ml
NaH ₂ PO ₄ · 2H ₂ O	2.14 mg/ml
NaCl	9.0 mg/ml

15 pH was adjusted to 6.1.

Example 10**Tibia test**

To estimate the in vivo biological potency of the hGH crystals prepared according to the invention a tibia test was performed
20 using hypophysectomized rats. The test was performed in accordance with the method described in the European Pharmacopoeia.

Two preparations of hGH crystals produced according to example 1 and formulated as preparations according to example 9 (F-7

and F-8) each containing an estimated amount equivalent to 4 IU were tested against a dissolved standard hGH preparation.

The following results were obtained:

Table 1

5 The potency of the preparations F-7 and F-8

	Test preparat.	Potency % of std.	IU/vial	95% confid. limits, % of std.
10	F-7	90.1	3.9	87.6 - 114.1
	F-8	103.8	4.5	90.6 - 110.4
15	Std. hGH 1986	≡ 100.0	≡ 4.4	-

From the performed test it can be concluded that the hGH crystals according to the invention are equally biological potent as the solubilized hGH standard and therefore will have
20 a bioavailability equal to that of usual solubilized hGH.

Example 11

hGH crystals were grown as described in example 5. Immediately before use a suspension was prepared by centrifugation of the crystals, subsequent removal of the motherfluid, and resuspen-
25 sion of the crystals in sterile 10 mM NaH₂PO₄, pH 6.2 giving a final concentration of 0.16 mg hGH/ml suspension.

The suspension was used to estimate the potency of the hGH crystal preparation in a weight gain assay. The test was performed in accordance with the method described in the
30 European Pharmacopoeia, with the exception that the time of dosing was prolonged to 10 days in order to optimize the biological response.

Two preparations of hGH crystals were used, each containing the same amount of hGH protein as the preparations of a growth hormone standard, which they were tested against. The standard was a reconstituted freeze-dried hGH preparation. All the 5 animals received the same amount of hGH.

The potency of the hGH crystal preparations were found to be 92.6% of the standard. The 95% confidence limits were 79.1 - 126.4% of the standard.

The hGH crystal preparation was thus shown to have a biological 10 potency equal to that of the solubilized hGH standard.

Example 12

Stability of hGH crystals stored in suspension for 6 months at 22-24°C.

The crystals were formed as described in Example 1 with the 15 exception that 7.5% (v/v) acetone was added instead of 10%.

The crystals were allowed to remain in suspension in the mother fluid for 6 months at 22-24°C. A sample of hGH crystals were removed by centrifugation, washed once with crystallization buffer without acetone and solubilized by raising the pH to 20 8.0.

The solubilized hGH crystals were subjected to analysis on ion exchange HPLC and GPC for detection of desamido and split forms or dimers and polymers, respectively.

When the data were compared with those of a reconstituted 25 lyophilized hGH preparation stored at 25°C for 32 days the content of the main peak of hGH in reconstituted hGH crystals was superior to reconstituted lyophilized hGH, stored under comparable conditions (see table 2).

Table 2

	Reconsti- tuted hGH	Crystals
Storage	25°C 32 days	22-24°C 6 months
Main peak on IE-HPLC (%)	71.2	92.3
5 Dimer (%)	0.7	1.2
Polymer (%)	0.3	0.3
Desamido (%)	25.9	5.0
Didesamido (%)	2.9	1.8
Split form (%)	-	-

CLAIMS.

1. A process for production of cation crystals of GH or of GH derivatives, comprising the following steps:
 - a) adding cations of inorganic or organic nature to a solution
5 of GH or derivatives thereof at a pH between 5 and 8,
 - b) growing of crystals at a temperature from about 0 to about 30°C, and
 - c) isolation of the cation crystals by known means.
2. A process according to claim 1, wherein the pH in step a) is
10 from 5.0 to 7.5, preferably 5.0 to 6.8.
3. A process according to claim 1, wherein the pH in step a) is below 7.
4. A process according to claim 1, wherein the pH in step a) is from 5.8 to 6.5, preferably from 6.0 to 6.5.
- 15 5. A process according to any of the previous claims, wherein an organic solvent or a mixture of organic solvents is added in step a).
6. A process according to claim 5, wherein the organic solvent is selected from the group consisting of short chained aliphatic, cyclic or aromatic alcohols or ketones.
20
7. A process according to claim 6, wherein the organic solvent is selected from the group consisting of acetone, methanol, ethanol and 2-propanol.
8. A process according to claim 7, wherein the organic solvent
25 is ethanol or acetone.

9. A process according to any of claims 5 to 8 wherein the organic solvent is added in a concentration of about 0.1 to about 50% v/v.
10. A process according to claim 9, wherein the organic solvent is added in a concentration of 0.1 to 30%, preferably from 0.1 to 20%, more preferred from 5 to 15% and most preferred from 6 to 12%.
11. A process according to any of the preceding claims 1 to 10, wherein the cation is a divalent cation.
- 10 12. A process according to claim 11, wherein the divalent cation is Zn^{++} .
13. A process according to claim 12, wherein Zn^{++} is added in a concentration below the limit for unspecific precipitation of amorpheus material.
- 15 14. A process according to claim 13, wherein Zn^{++} is added in a concentration from 0.5 to 10 mol Zn^{++} /mol GH.
15. A process according to claim 14 wherein the concentration of Zn^{++} is from 1.0 to 3.0 mol Zn^{++} /mol GH, more preferred from 1.1 to 2.2 mol Zn^{++} /mol GH and most preferred from 1.2 to 2.0
- 20 mol Zn^{++} /mol GH.
16. A process according to any of the preceding claims, wherein the growth hormone is hGH or derivatives thereof.
17. A process according to any of the preceding claims, wherein the temperature in step b) is from about 4 to about 25°C.
- 25 18. Cation crystals of GH or GH derivatives.
19. Crystals according to claim 17, wherein the growth hormone is hGH or derivatives thereof.

20. Crystals according to claims 17 or 18, wherein the cation is Zn^{++} .

21. Crystals according to claim 19, wherein the molar ratio between Zn^{++} and GH is from about 0.2 to about 10, preferably 5 from about 0.5 to 5 and more preferably from about 0.5 to 2.0.

22. Pharmaceutical preparations, characterized in that they contain crystals according to any of claims 16 to 20.

23. Use of a crystallization process according to claims 1 to 15 as a purification and/or isolation step in the manufacturing of GH.

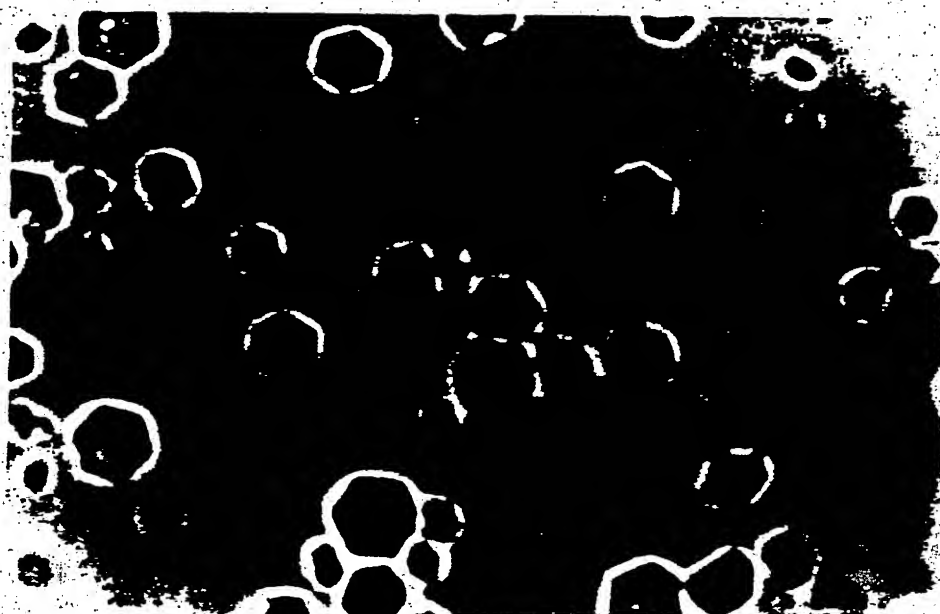


Fig. 1

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 91/00203

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁵ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: C 07 K 13/00, A 61 K 37/36		
II. FIELDS SEARCHED Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
IPC5	A 61 K; C 07 K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched ⁸		
SE,DK,FI,NO classes as above		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	Chemical Abstracts, volume 106, no. 25, 22 June 1987, (Columbus, Ohio, US), Spitsberg, Vitaly L.: "A selective extraction of growth hormone from bovine pituitary gland and its further purification and crystallization ", see page 78, abstract 207835d, & Anal. Biochem. 1987, 160(2), 489- 495	1-23
A	Chemical Abstracts, volume 103, no. 9, 2 September 1985, (Columbus, Ohio, US), Bell, Jeffrey A et al.: "Crystallization and preliminary x-ray characteri-zation of bovine growth hormone. Purification of bovine prolactin and growth hormone ", see page 80, abstract 65105c, & J. Biol. Chem. 1985, 260(14), 8520-8525	1-23
* Special categories of cited documents:¹⁰ "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Z" document member of the same patent family		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
21st October 1991		1991 -10- 2 4
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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Chemical Abstracts, volume 106, no. 22, 1 June 1987, (Columbus, Ohio, US), see page 413, abstract 182 702t, & JP, A, 6248635 (Sustained-release formulation containing growth hormone) 1987 --	1-23
A	EP, A2, 0355460 (AMERICAN CYANAMID COMPANY) 28 February 1990, see the whole document --	1-23
A	EP, A2, 0343696 (MONSANTO COMPANY) 29 November 1989, see the whole document --	1-23
A	EP, A2, 0177478 (MONSANTO COMPANY) 9 April 1986, see the whole document --	1-23
A	EP, A1, 0216485 (INTERNATIONAL MINERALS AND CHEMICAL CORPORATION) 1 April 1987, see the whole document --	1-23
A	EP, A2, 0277043 (INTERNATIONAL MINERALS AND CHEMICAL CORPORATION) 3 August 1988, see the whole document -- -----	1-23

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 91/00203**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 91-09-27. The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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